

Control of Signal Transduction Cycles: General Results and Application to the Jak-Stat Pathway

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Abstract

Signal transduction involves the transitions of proteins between inactive and active states that can be achieved by reversible phosphorylation, nucleo-cytoplasmic transport, and other processes. We consider a network of such state transitions governed by first-order kinetics and analyse how the reactions control the occupancy of the network states. First, a theorem is derived that relates concentration control coefficients and occupancy of the network states. Second, it is shown that the absolute value of each control coefficient is bounded by unity, so that the network does not exhibit ultrasensitive responses. Third, the signs of certain control coefficients are derived from the network topology. These results are applied to a mathematical model of the Jak/Stat1 signaling. This pathway has been thought to function as a continuous cycle of cytoplasmic activation, nuclear import, inactivation and re-export of Stat1 transcription factors, but the recent discovery of an apparently futile nucleo-cytoplasmic cycle of inactive Stat1 has yielded a more complex picture. We demonstrate here two consequences of shuttling: (1) homeostasis of unphosphorylated Stat1 in the cell nucleus and (2) enhanced stimulus sensitivity of the pathway, and discuss their functional implications.

Keywords: Stat pathway, concentration control coefficient, summation theorem, node control, homeostasis, sensitivity

1 Introduction

Signal transduction processes proceed through the reversible activation of proteins such as by phosphorylation. While one typically distinguishes between an inactive and an active state of a given signaling protein, the state transitions can be far more complex. They can involve multiple phosphorylations, other covalent modifications, formation of protein complexes, and transports between different cellular compartments. A much studied example are the cytokine/Jak/Stat pathways. Following tyrosine phosphorylation by the Jak kinases associated with cytokine receptors, the Stat (*S*ignal transducer and *a*ctivator of *t*ranscription) transcription factors gain access to the nucleus and thus their target genes [10]. This process is regulated by a further phosphorylation on a serine residue [8].

The network organization of signaling processes raises the question of how individual processes (such as phosphorylation, nuclear import, dephosphorylation) control the biological activity of the protein in question. The impact of a particular process can depend both on the network topology and the kinetic parameters of this step and the other processes in the network. In this paper, we analyse networks that describe the state transitions of signaling proteins and derive several general results of how control is distributed among the individual transition steps. These results are then applied to investigate the structural design of the Jak/Stat pathway.

2 Network of State Transitions

We consider a signaling protein that can be in N different states. Describing the state transitions by linear rate laws, we obtain for the fractions of the various states X_i

$$\dot{X}_i = \sum_j k_{ji} X_j - \sum_j k_{ij} X_i, \quad i = 1, \dots, N, \quad (1)$$

where mass conservation implies $\sum_i X_i = 1$ (i.e., the X_i are fractions of total number of molecules). k_{ij} denotes the rate constant of transition from state i to state j . Note that the same form of kinetic equations has been used for simple models of gene-regulatory networks [3], and is a common description for ion channel gating [7].

2.1 Steady State

The steady state is found by solving the linear system

$$\sum_j k_{ji} \bar{X}_j - \sum_j k_{ij} \bar{X}_i = 0. \quad (2)$$

When solving for the steady state concentration \bar{X}_m , it is convenient to replace the m th equation with the conservation relation:

$$\begin{aligned} -\sum k_{1j} \bar{X}_1 + k_{21} \bar{X}_2 + \dots + k_{m1} \bar{X}_m + \dots + k_{N1} \bar{X}_N &= 0 \\ &\vdots \\ \bar{X}_1 + \bar{X}_2 + \dots + \bar{X}_m + \dots + \bar{X}_N &= 1 \\ &\vdots \\ k_{1N} \bar{X}_1 + k_{2N} \bar{X}_2 + \dots + k_{mN} \bar{X}_m + \dots + -\sum k_{nj} \bar{X}_N &= 0 \end{aligned} \quad (3)$$

A similar linear problem occurs in the derivation of enzymatic rate laws by using the quasi-steady state approximation. In the following treatment we employ several arguments that have originally been developed by King and Altman in this context [4, 6]. Cramer's rule applied to Eq. (3) yields

$$\bar{X}_m = \frac{N_m}{D} = \frac{|M^m|}{|M|}, \quad (4)$$

where

$$|M^m| = \begin{vmatrix} -\sum k_{1j} & \dots & 0 & \dots & k_{N1} \\ \vdots & & \vdots & & \vdots \\ 1 & \dots & 1 & \dots & 1 \\ \vdots & & \vdots & & \vdots \\ k_{1N} & \dots & 0 & \dots & -\sum k_{Nj} \end{vmatrix} \quad \text{and} \quad |M| = \begin{vmatrix} -\sum k_{1j} & \dots & k_{m1} & \dots & k_{N1} \\ \vdots & & \vdots & & \vdots \\ 1 & \dots & 1 & \dots & 1 \\ \vdots & & \vdots & & \vdots \\ k_{1N} & \dots & k_{mN} & \dots & -\sum k_{Nj} \end{vmatrix}. \quad (5)$$

By application of $(m-1)$ switches of adjacent rows, the m -th row of M^m consisting entirely of ones can be brought in the first row. Likewise, $(m-1)$ switches of adjacent columns in M^m bring the m -th column containing the zeros in the first position. Therefore it follows for the numerator $N_m = |M^m|$

$$N_m = (-1)^{2(m-1)} \begin{vmatrix} 1 & 1 & 1 & \dots & 1 \\ 0 & -\sum k_{1j} & k_{21} & \dots & k_{N1} \\ \vdots & \vdots & \vdots & & \vdots \\ 0 & k_{1N} & k_{2N} & \dots & -\sum k_{Nj} \end{vmatrix}$$

$$= \begin{vmatrix} -\sum k_{1j} & k_{21} & \dots & k_{N1} \\ \vdots & \vdots & & \vdots \\ k_{1N} & k_{2N} & \dots & -\sum k_{Nj} \end{vmatrix}. \quad (6)$$

Inspection of (6) and (4) shows that the numerator N_m has the following properties (see [4]):

(I) Because $\sum_i X_i = 1$ it follows immediately that

$$\sum_i N_i = D. \quad (7)$$

(II) It can be shown that the expansion of the determinant $|M^m|$ is a sum of products with the same sign, and this sign is the same for all m . The determinant is positive if $(N - 1)$ is even and negative otherwise. For odd $(N - 1)$ each N_m can be multiplied by -1 without changing the value of $\frac{N_m}{D}$; therefore N_m can always be chosen to be positive.

(III) Each term in the expansion of $|M^m|$ is a product which contains exactly one matrix element M_{ij}^m from each column j . After some algebra, one finds, moreover, that the sum obtained from the expansion of $|M^m|$ contains each k_{ij} for each i exactly once. Thus N_m can always be expressed as

$$N_m = \sum_j k_{ij} \frac{\partial N_m}{\partial k_{ij}} \text{ for all } i \neq m. \quad (8)$$

(IV) $|M^m|$ contains no constants k_{mi} with m as the first index. Hence

$$\frac{\partial N_m}{\partial k_{mi}} = 0 \text{ for all } i. \quad (9)$$

2.2 Auxiliary Inequalities

In this paragraph we will derive inequalities for the numerator N_m and denominator D used to calculate the steady-state concentrations \bar{X}_m with the help of Eq. (4). These will turn out useful later. With property (II) and the fact that all $k_{ij} \geq 0$ follows

$$\frac{\partial N_m}{\partial k_{ij}} \geq 0 \text{ for all } i, j, m \quad (10)$$

From (III) and (10) we have

$$\frac{\partial N_m}{\partial k_{ij}} k_{ij} \leq N_m \text{ for all } i, j, m, \quad (11)$$

implying that (with Eq. (7))

$$\frac{\partial D}{\partial k_{ij}} k_{ij} = \sum_l \frac{\partial N_l}{\partial k_{ij}} k_{ij} \leq D \text{ for all } i, j. \quad (12)$$

The method by King and Altman can be used to calculate the steady state (4) in an algorithmic way from the topology of the network, without explicitly evaluating the determinants $|M^m|$ and $|M|$. The algorithm is based on the concept of allowed sub-paths of the network. Since this concept will be used later, we briefly summarize the requirements for an allowed sub-path S_m (see [4, 6]): (1) An allowed sub-path S_m contains $(N - 1)$ edges of the network; (2) obeys the direction of these edges; (3) visits every network node exactly once (i.e. the sub-path contains no loops) and (4) ends exclusively at node m . Each allowed sub-path is then interpreted as a product of the rate constants k_{ij} corresponding to

the edges in the sub-path and $|M^m| = N_m$ is the sum of the products of all allowed sub-paths S_m . The denominator $|M| = D$ can then easily be calculated from (7).

Using the King-Altman algorithm, it can be shown that the expression for D must contain each k_{ij} at least once; since D is a sum of positive products (cf. (II)) we have

$$\frac{\partial D}{\partial k_{ij}} > 0 \text{ for all } i, j. \quad (13)$$

2.3 Concentration Control Coefficients

The concentration control coefficient C_{ij}^m measures the relative change of the concentration \bar{X}_m caused by a relative change of a rate constant k_{ij} [5]. Using Eq. (4), C_{ij}^m can be calculated as follows:

$$C_{ij}^m = \frac{k_{ij}}{\bar{X}_m} \frac{\partial \bar{X}_m}{\partial k_{ij}} = \frac{k_{ij}}{N_m} D \frac{\partial(N_m/D)}{\partial k_{ij}} = \frac{k_{ij}}{N_m} \frac{\partial N_m}{\partial k_{ij}} - \frac{k_{ij}}{D} \frac{\partial D}{\partial k_{ij}}. \quad (14)$$

2.3.1 Control of Network Nodes

We define the following sum

$$\hat{C}_i^m = \sum_j C_{ij}^m = \sum_j \frac{k_{ij}}{N_m} \frac{\partial N_m}{\partial k_{ij}} - \sum_j \frac{k_{ij}}{D} \frac{\partial D}{\partial k_{ij}} \quad (15)$$

that measures the total control of the processes emanating from node i on the concentration of state m . For the first sum one can write

$$\begin{aligned} \sum_j \frac{k_{ij}}{N_m} \frac{\partial N_m}{\partial k_{ij}} &= \begin{cases} \frac{1}{N_m} \sum_j k_{mj} \frac{\partial N_m}{\partial k_{mj}} = 0 & \text{for } i = m \text{ (cf Eq. 9)} \\ \frac{1}{N_m} \sum_j k_{ij} \frac{\partial N_m}{\partial k_{ij}} = 1 & \text{for } i \neq m \text{ (cf Eq. 8)} \end{cases} \\ &= 1 - \delta_{im}, \end{aligned}$$

where δ_{im} denotes the Kronecker symbol. Using Eqs (7), (8) and (9) again, the second sum in Eq. (15) can be expressed as

$$\begin{aligned} \sum_j \frac{k_{ij}}{D} \frac{\partial D}{\partial k_{ij}} &= \frac{1}{D} \sum_j k_{ij} \frac{\partial(\sum_l N_l)}{\partial k_{ij}} = \frac{1}{D} \sum_l \sum_j k_{ij} \frac{\partial N_l}{\partial k_{ij}} \\ &= \frac{1}{D} \sum_{l \neq i} N_l = \frac{D - N_i}{D} = 1 - \bar{X}_i. \end{aligned} \quad (16)$$

Taken together, this yields for the control of node i on \bar{X}_m a new summation theorem

$$\begin{aligned} \hat{C}_i^m = \sum_j C_{ij}^m &= \begin{cases} \bar{X}_m - 1 & \text{for } i = m \\ \bar{X}_i & \text{for } i \neq m \end{cases} \\ &= \bar{X}_i - \delta_{im}. \end{aligned} \quad (17)$$

Note that by summing over all nodes i one immediately obtains the summation theorem for concentration control coefficients of metabolic control theory, $\sum_{i,j} C_{ij}^m = 0$ [5].

Eq. (17) implies that the total control of a node i (i.e., the sum of the control coefficients of all reactions for which X_i is a substrate) onto the concentration at a different node X_m is equal to the steady-state concentration at node i . The total control of a node on its own concentration (i.e. $i = m$) is equal to the negative sum of the steady state concentrations of the remaining compounds. These results imply that the control of a reaction which is the only outgoing edge from a specific node can be determined experimentally by measuring the relative steady-state concentration of that node.

2.3.2 Concentration Control is Bounded by Unity

Since N_m is a positive sum of products of the k_{ij} (see property (II) in Section 2.1) it follows from Eq. (8) that

$$0 \leq k_{ij} \frac{\partial N_m}{\partial k_{ij}} \leq N_m \quad (18)$$

therefore

$$0 \leq \frac{k_{ij}}{N_m} \frac{\partial N_m}{\partial k_{ij}} \leq 1 \quad \text{and} \quad 0 \leq \frac{k_{ij}}{D} \frac{\partial D}{\partial k_{ij}} = \frac{1}{D} \sum_l k_{ij} \frac{\partial N_l}{\partial k_{ij}} \leq 1.$$

Then the minimum of the concentration control coefficient is given by

$$\min C_{ij}^m = \min \left(\frac{k_{ij}}{N_m} \frac{\partial N_m}{\partial k_{ij}} - \frac{k_{ij}}{D} \frac{\partial D}{\partial k_{ij}} \right) = -1 \quad (19)$$

and the maximum control is

$$\max C_{ij}^m = \max \left(\frac{k_{ij}}{N_m} \frac{\partial N_m}{\partial k_{ij}} - \frac{k_{ij}}{D} \frac{\partial D}{\partial k_{ij}} \right) = 1. \quad (20)$$

It follows as a general property of linear mass conserved networks that the control of all edges is never ultra-sensitive:

$$|C_{ij}^m| \leq 1. \quad (21)$$

2.4 Positive or Negative Control?

Whether a given edge in a network has a positive or negative control on a specific state may depend on the rate constants k_{ij} . However, under certain conditions the sign of concentration control coefficients can be deduced solely from the network topology.

2.4.1 Steps with Negative Control

Since N_m is positive (see property (II) in Section 2.1) the sign of

$$C_{ij}^m = \frac{k_{ij}}{N_m} \left(\frac{\partial N_m}{\partial k_{ij}} - \frac{N_m}{D} \frac{\partial D}{\partial k_{ij}} \right) \quad (22)$$

is determined by the term in brackets. According to Eq. (13) $\partial D / \partial k_{ij}$ is always positive. If in a given network *no* allowed sub-path leading to node m contains the reaction k_{ij} it follows immediately that $\partial N_m / \partial k_{ij} = 0$ and therefore

$$C_{ij}^m < 0. \quad (23)$$

2.4.2 Steps with Positive Control

If in a given network *all* allowed sub-paths leading to node m contain the edge k_{ij} then

$$\frac{\partial N_m}{\partial k_{ij}} k_{ij} = N_m \quad (24)$$

and

$$\frac{\partial N_m}{\partial k_{ij}} - \frac{N_m}{D} \frac{\partial D}{\partial k_{ij}} = \frac{\partial N_m}{\partial k_{ij}} - \frac{\frac{\partial N_m}{\partial k_{ij}} k_{ij}}{D} \frac{\partial D}{\partial k_{ij}} = \frac{\partial N_m}{\partial k_{ij}} \left(1 - \frac{1}{D} \frac{\partial D}{\partial k_{ij}} \right). \quad (25)$$

Substituting Eqs (12) and (10) into (25) yields

$$C_{ij}^m > 0. \quad (26)$$

Furthermore, if all allowed sub paths of the network through the edge k_{ij} lead exclusively to node m , it follows from (7) that

$$\frac{\partial D}{\partial k_{ij}} = \frac{\partial N_m}{\partial k_{ij}}. \quad (27)$$

Then we have for

$$\frac{\partial N_m}{\partial k_{ij}} - \frac{N_m}{D} \frac{\partial D}{\partial k_{ij}} = \frac{\partial N_m}{\partial k_{ij}} - \frac{N_m}{D} \frac{\partial N_m}{\partial k_{ij}} = \frac{\partial N_m}{\partial k_{ij}} \left(1 - \frac{N_m}{D}\right),$$

where $N_m/D < 1$. From (10), it follows that for such a reaction k_{ij}

$$C_{ij}^m > 0. \quad (28)$$

3 Application to the Jak/Stat Signal Transduction Pathway

Stat transcription factors regulate cell growth, proliferation, immune responses, and other processes. The stimulation of cytokine receptors causes the tyrosine phosphorylation of cytoplasmic Stats through receptor-bound Jak kinases. Phosphorylated Stat dimers are imported into the cell nucleus where they regulate the expression of their target genes. After dephosphorylation, the inactive Stat molecules are transported back into the cytoplasm where they can undergo a further round of phosphorylation, nuclear import etc. [10]. In the following, we apply the above theoretical results to study the control of this signal transduction pathway.

3.1 Linear Stat1 Model

We have developed a mathematical model of the interferon/Stat1 signaling pathway [1]. This model describes the dynamics of the inactive and active cytokine receptor and of the phosphorylated and unphosphorylated Stat1 fractions in the cytoplasm and in the nucleus and is able to reproduce experimental data from different cell types and for various stimulation conditions. The nonlinear model can be reduced by eliminating fast protein-protein interactions through rapid-equilibrium approximations to the following linear core model (Fig. 1A):

$$\begin{aligned} \dot{S}_1 &= k_{41} S_4 - (k_{12} + k_{14}) S_1 & \dot{S}_3 &= k_{23} S_2 - k_{34} S_3 \\ \dot{S}_2 &= k_{12} S_1 - k_{23} S_2 & \dot{S}_4 &= k_{34} S_3 + k_{14} S_1 - k_{41} S_4. \end{aligned} \quad (29)$$

The variables S_i denote the following Stat1 fractions: S_1 – cytoplasmic unphosphorylated, S_2 – cytoplasmic phosphorylated, S_3 – nuclear phosphorylated, and S_4 – nuclear unphosphorylated. Mass conservation implies $S_1 + S_2 + S_3 + S_4 = 1$. The first-order rate constants are assigned to the following reactions:

$$\begin{aligned} k_{12} &: \text{phosphorylation} & k_{23} &: \text{nuclear import of phospho-Stat1} \\ k_{34} &: \text{effective dephosphorylation} & k_{14} &: \text{nuclear import of unphosphorylated Stat1} \\ k_{41} &: \text{nuclear export} & & \end{aligned} \quad (30)$$

For simplicity, we assume equal nuclear and cytoplasmic volumes.

The steady state of Eqs (29) is

$$\begin{aligned} \bar{S}_1 &= \frac{1}{T} \frac{1}{k_{12}} & \bar{S}_3 &= \frac{1}{T} \frac{1}{k_{34}} \\ \bar{S}_2 &= \frac{1}{T} \frac{1}{k_{23}} & \bar{S}_4 &= \frac{1}{T} \frac{1}{k_{41}} \left(1 + \frac{k_{14}}{k_{12}}\right) \end{aligned} \quad (31)$$

with

$$T = \frac{1}{k_{12}} + \frac{1}{k_{23}} + \frac{1}{k_{34}} + \frac{1}{k_{41}} \left(1 + \frac{k_{14}}{k_{12}}\right). \quad (32)$$

3.2 Control Analysis

While the input stimuli of Jak/Stat pathways are transient, they are typically present sufficiently long for the pathway to reach a steady state. For example, cytokine signals between cells of the immune system are limited to certain phases of the immune responses, lasting between one and several hours. For the experimentally estimated parameters, the steady state is reached within less than 30 minutes, so that the steady state solution provides an appropriate measure of the response amplitude. Eq. (29) thus can be used to characterize how the response in terms of nuclear phospho-Stat1 depends on the parameters of the individual reaction and transport processes in the cycle. This can be done systematically by using the concentration control coefficients introduced in the previous section. Applying the node control summation rule, Eq. (17), three control coefficients for nuclear phospho-Stat1 S_3 can be directly read off the reaction scheme in Fig. 1A:

$$\begin{aligned} C_{23}^3 &= \overline{S_2} &= \frac{1}{T} \frac{1}{k_{23}} \\ C_{34}^3 &= \overline{S_3} - 1 &= -\frac{1}{T} \left(\frac{1}{k_{12}} + \frac{1}{k_{23}} + \frac{1}{k_{41}} \left(1 + \frac{k_{14}}{k_{12}}\right) \right) \\ C_{41}^3 &= \overline{S_4} &= \frac{1}{T} \frac{1}{k_{41}} \left(1 + \frac{k_{14}}{k_{12}}\right) \end{aligned} \quad (33)$$

For the kinase reaction and the nuclear import process of unphosphorylated Stat1 we have

$$C_{12}^3 + C_{14}^3 = \overline{S_1} = \frac{1}{T} \frac{1}{k_{12}} \quad (34)$$

where

$$C_{12}^3 = \frac{1}{T} \frac{1}{k_{12}} \left(1 + \frac{k_{14}}{k_{41}}\right) \quad C_{14}^3 = -\frac{1}{T} \frac{k_{14}}{k_{12}k_{41}}. \quad (35)$$

The expressions for the control coefficients show that each coefficient retains the same sign for all values of the kinetic parameters. This implies that whether a process exerts a positive or a negative effect on the nuclear accumulation of phospho-Stat1 depends on its position and direction in the cycle but not on the specific parameters. Phosphorylation, nuclear import of the phospho-protein and nuclear export always exert positive control (C_{12}^3 , C_{23}^3 and $C_{41}^3 > 0$), while nuclear dephosphorylation and import of the unphosphorylated species have negative control (C_{34}^3 and $C_{14}^3 < 0$). Furthermore, it can be shown that C_{34}^3 has always the largest absolute value of all control coefficients. Therefore, nuclear dephosphorylation is the step with the strongest control independently of the kinetic parameter values.

3.3 Effects of Shuttling

The discovery of an import pathway for unphosphorylated Stat1 (rate k_{14} in Fig. 1A) has raised the question of its functional significance. In the cycle of Stat1 activation/inactivation, this step would seem dispensable; instead the pathway design shown in Fig. 1B has originally been anticipated [9]. This import step would ensure the nuclear presence of the unphosphorylated molecule, which may serve specific functions [2, 11].

However, we have found that the continuous nucleo-cytoplasmic shuttling of unphosphorylated Stat1 that proceeds through the steps with rate constants k_{41} and k_{14} has critical implications for the regulation of both the unphosphorylated and phosphorylated Stat1. To demonstrate this, we will compare the wild-type pathway design (Fig. 1A) with the hypothetical pathway shown in Fig. 1B, in

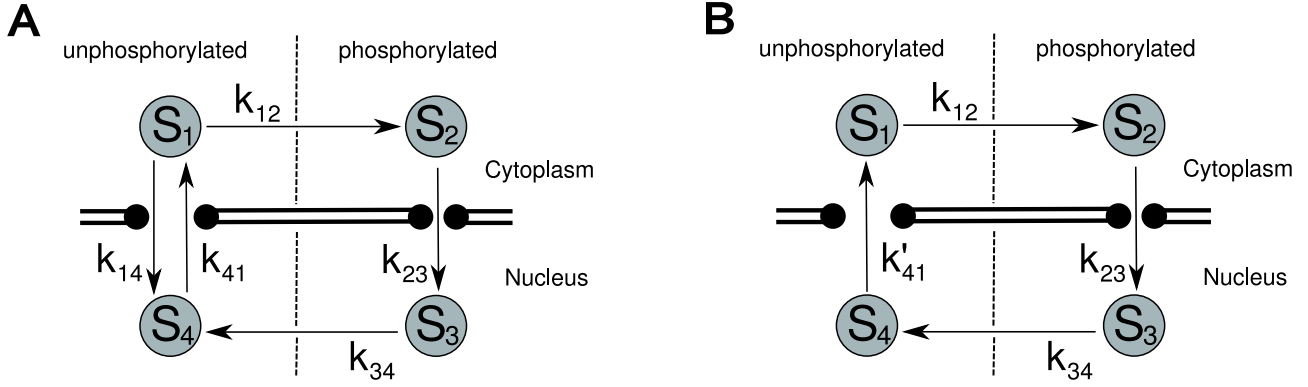


Figure 1: Reaction scheme of the core Stat1 model for the wild-type system (A) and the hypothetical ΔI mutant (B).

which nuclear import of unphosphorylated Stat1, and thus nucleo-cytoplasmic shuttling, do not occur. The hypothetical pathway, which will be referred to as ΔI , has the same steady state as the wild-type pathway, when the export rate constant k_{41} is adjusted to the new value

$$k'_{41} = k_{41} \frac{k_{12}}{k_{12} + k_{14}} \quad (36)$$

(see Eqs 29).

3.3.1 Homeostasis of Unphosphorylated Stat1 in the Nucleus

The summation rule for the node concentration control (17) allows for a general comparison of the control of specific steps in the hypothetical and wild-type Stat pathways. Since both pathways exhibit an identical steady state \bar{S} the sum at one node S_i , $\hat{C}_i^m = \sum_j C_{ij}^m = \bar{S}_i - \delta_{im}$, must yield the same value in both systems.

If the control of the receptor-associated Jak kinase k_{12} on the concentration of unphosphorylated nuclear Stat S_4 is considered, the above summation rule for the node S_1 in the hypothetical ΔI pathway lacking shuttling evaluates to $\hat{C}_1^4 = C_{12}^4$. In the wild-type system the corresponding node control is given by $\hat{C}_1^4 = C_{12}^4 + C_{14}^4$, taking into account the additional control of the shuttling process k_{14} .

The sign rule of Section 2.4.2 states that the control of the import process k_{14} on S_4 must be positive: $C_{14}^4 > 0$. Since \hat{C}_1^4 has the same value in both systems, it follows immediately that the import step in the wild-type pathway decreases the control of the receptor tyrosine kinase on S_4 . Analytical evaluation of C_{14}^4 shows that the control of the Jak kinase is reduced by

$$\Delta C_{12}^4 = C_{14}^4 = \frac{k_{14}}{k_{12} + k_{14}} - \frac{k_{14}}{k_{41}} S_1 \quad (37)$$

By writing the kinase control coefficient for the wild-type system as a function of the reaction rates k_{ij}

$$C_{12}^4 = \frac{\frac{1}{k_{12} + k_{14}} \left(1 + \frac{k_{12}}{k_{23}} + \frac{k_{12}}{k_{34}} \right) - \frac{1}{k_{23}} - \frac{1}{k_{34}}}{\frac{1}{k_{12}} + \frac{1}{k_{23}} + \frac{1}{k_{34}} + \frac{1}{k_{41}} + \frac{k_{14}}{k_{12} k_{41}}} \quad (38)$$

one can verify that the kinase control on the unphosphorylated nuclear Stat pool can get arbitrarily small if the shuttling rate k_{14} is large compared to the remaining transport and reaction rates.

Due to the decreased control of the receptor-associated kinase on S_4 , variations in the amount of activated receptor caused by cytokine stimulation of different strengths do not result in pronounced

fluctuations of the nuclear concentration of unphosphorylated Stat. As mentioned above, unphosphorylated Stat molecules have been reported to play an essential role in the regulation of several cytokine-independent genes [2, 11], therefore a relative robustness of the unphosphorylated nuclear Stat pool to varying stimulation strengths of the cytokine receptors might be essential to avoid crosstalk between different extracellular signals. A tightly balanced interplay of the export and import pathways might help the cell to decouple the regulation of different target gene groups.

3.3.2 Shuttling Increases Response Control of Receptor

Similar to the above analysis of the stabilizing effect of the import pathway on the concentration of nuclear unphosphorylated Stat1 one can investigate how nucleo-cytoplasmic shuttling affects the primary pathway response, that is the concentration change of transcriptional active phospho-Stat1 caused by cytokine stimulation.

In the hypothetical ΔI pathway without nuclear import of unphosphorylated Stat the node control of S_1 on the nuclear phospho-Stat1 dimer pool is determined solely by the control of the kinase step: $\hat{C}_1^3 = C_{12}^3$.

For the wild-type pathway the corresponding expression for the node control is $\hat{C}_1^3 = C_{12}^3 + C_{14}^3$ showing that the total control is distributed over the phosphorylation and import process.

Since the import step is not part of any allowed sub-path leading to S_3 (see Section 2.2), it follows from the sign rule in Section 2.4.1 that the control of k_{14} on the nuclear concentration of phosphorylated Stat1 is negative: $C_{14}^3 < 0$. Analogously to the previous section, in both signal transduction systems \hat{C}_1^3 must be identical, thus the node control summation rule implies that the shuttling process in the wild-type pathway increases the control of the Jak kinase by:

$$\Delta C_{12}^3 = |C_{14}^3| = \frac{k_{14}}{k_{41}} S_1 \quad (39)$$

Evaluating C_{12}^3 in the wild-type system as a function of the rate constants k_{ij}

$$C_{12}^3 = \frac{\frac{1}{k_{12}} + \frac{k_{14}}{k_{12} k_{41}}}{\frac{1}{k_{12}} + \frac{1}{k_{23}} + \frac{1}{k_{34}} + \frac{1}{k_{41}} + \frac{k_{14}}{k_{12} k_{41}}} \quad (40)$$

shows that the control approaches its maximal value 1 when the import k_{14} is made large compared with the other rate constants.

This results reveal that the shuttling of the inactive molecules makes the wild-type Stat pathway more sensitive to variations in receptor activation, increasing the response of the system to small changes in the extracellular ligand concentration.

4 Discussion

For the state-transition networks considered in this paper, we have demonstrated a relation between the fraction of a state m and the total control of all the steps emanating from this node. Interestingly, the total control exerted on *any* other state $i \neq m$ in the network is equal to the the steady-state fraction \overline{X}_m . Thus, if only a single process emanates from node m , the control of this process simply equals \overline{X}_m . However, when several steps emanate from node m , some (or all) of them can have high control even when \overline{X}_m is small. This is the case when there are control coefficients with different signs, that can partially cancel each other in the summation in Eq. (17). It remains an interesting area for further study whether such general approaches can be extended to study the control distribution in more complex signaling networks.

These general results derived here have proved very useful in the study of the Jak/Stat signal transduction pathway. Here the experimental finding that unphosphorylated – and thus inactive – Stat

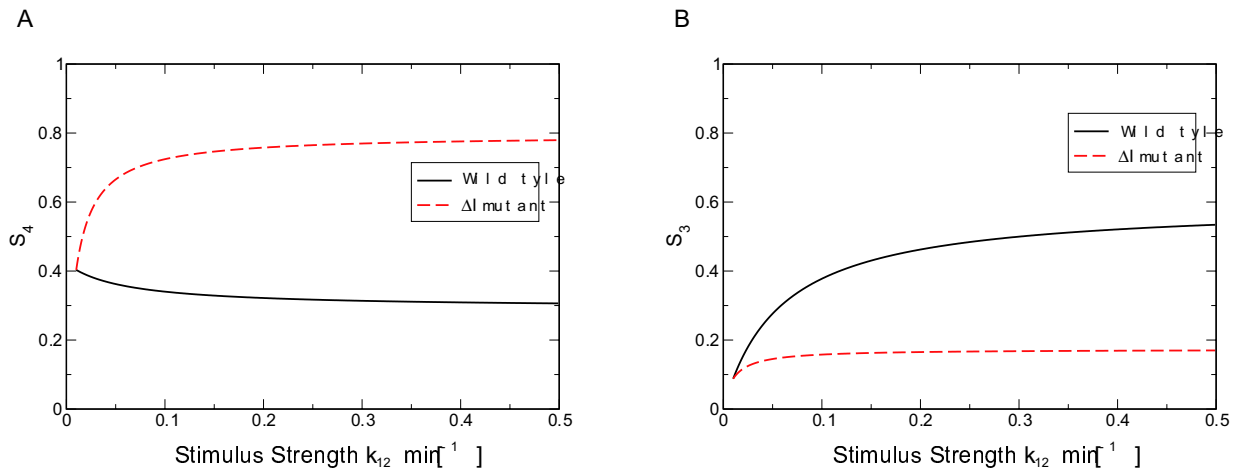


Figure 2: Stimulus-Response curves for the wild-type system and the hypothetical ΔI mutant. Shown is the concentration of S_4 (A) and S_3 (B) as a function of the phosphorylation rate k_{12} . For illustrative purposes, we chose parameter values derived from the more detailed model parameters, which were estimated from experimental data [1]: $k_{23} = 0.3$, $k_{34} = 0.06$, $k_{41} = 0.11$, $k_{14} = 0.08$ in units min^{-1} . The reduced export rate k'_{41} of the ΔI mutant was matched at the reference phosphorylation rate $k_{12} = 0.01$.

transcription factors (such as Stat1 and Stat3) are rapidly imported into the cell nucleus has initially been puzzling [10]. Because unphosphorylated Stats are exported from the nucleus, this import leads to an apparently futile nucleo-cytoplasmic shuttling. We have demonstrated that shuttling can have significant functional consequences. It strongly increases the control exerted by the cytokine stimulus on the nuclear accumulation of transcriptionally active, phosphorylated Stats and thus extends the response range of the pathway. Moreover, it has recently been found that also unphosphorylated Stats may serve nuclear functions [11]. Interestingly, nucleo-cytoplasmic shuttling makes the nuclear fraction of unphosphorylated Stats insensitive to cytokine stimulation and subsequent Stat phosphorylation. In this way, the regulation of the conventional phospho-Stat target genes could be uncoupled from processes mediated by unphosphorylated Stats.

Acknowledgments

We acknowledge the stimulating collaboration with Dr. Uwe Vinkemeier and Dr. Thomas Meyer (Leibniz Institute for Molecular Pharmacology Berlin) on interferon/Stat signaling.

References

- [1] Beirer, S., Meyer, T., Vinkemeier, U., and Höfer, T., An experimentally-based mathematical model of the IFN γ -Stat1 signaling pathway, *Preprint*.
- [2] Chatterjee-Kishore, M., Wright, K.L., Ting, J.P., and Stark, G.R., How Stat1 mediates constitutive gene expression: a complex of unphosphorylated Stat1 and IRF1 supports transcription of the LMP2 gene, *EMBO J.*, 19:4111–4122, 2000.
- [3] Chen, T., He, H.L., and Church, G.M., Modeling gene expression with differential equations, *Pacific Symp. Biocomputing '99*, World Scientific, 29–40, 1999.
- [4] Cornish-Bowden, A., *Fundamentals of Enzyme Kinetics*, Portland Press, 1995.

- [5] Heinrich, R. and Schuster, S., *The Regulation of Cellular Systems*, Chapman and Hall, 1996.
- [6] King, E. L. and Altman, C., A schematic method of deriving the rate laws for enzyme-catalyzed reactions, *J. Phys. Chem.*, 60:1375–1378, 1956.
- [7] Koch, C. and Segev, I. Eds, *Methods in Neuronal Modeling: From Ions to Networks*, Bradford Book MIT Press, 1998.
- [8] Lödige, I., Marg, A., Wiesner, B., Malecova, B., Oelgeschlager, T., and Vinkemeier, U., Nuclear export determines the cytokine sensitivity of STAT transcription factors, *J. Biol. Chem.*, 280:43087–43099, 2005.
- [9] Swameye, I., Müller, T.G., Timmer, J., Sandra, O., and Klingmüller, U., Identification of nucleocytoplasmic cycling as a remote sensor in cellular signaling by databased modeling, *Proc. Natl. Acad. Sci. USA*, 100:1028–1033, 2003.
- [10] Vinkemeier, U., Getting the message across, STAT! Design principles of a molecular signaling circuit, *J. Cell Biol.*, 167:197–201, 2004.
- [11] Yang, J., Chatterjee-Kishore, M., Staugaitis, S.M., Nguyen, H., Schlessinger, K., Levy, D.E., and Stark, G.R., Novel roles of unphosphorylated STAT3 in oncogenesis and transcriptional regulation, *Cancer Res.*, 65:939–947, 2005.